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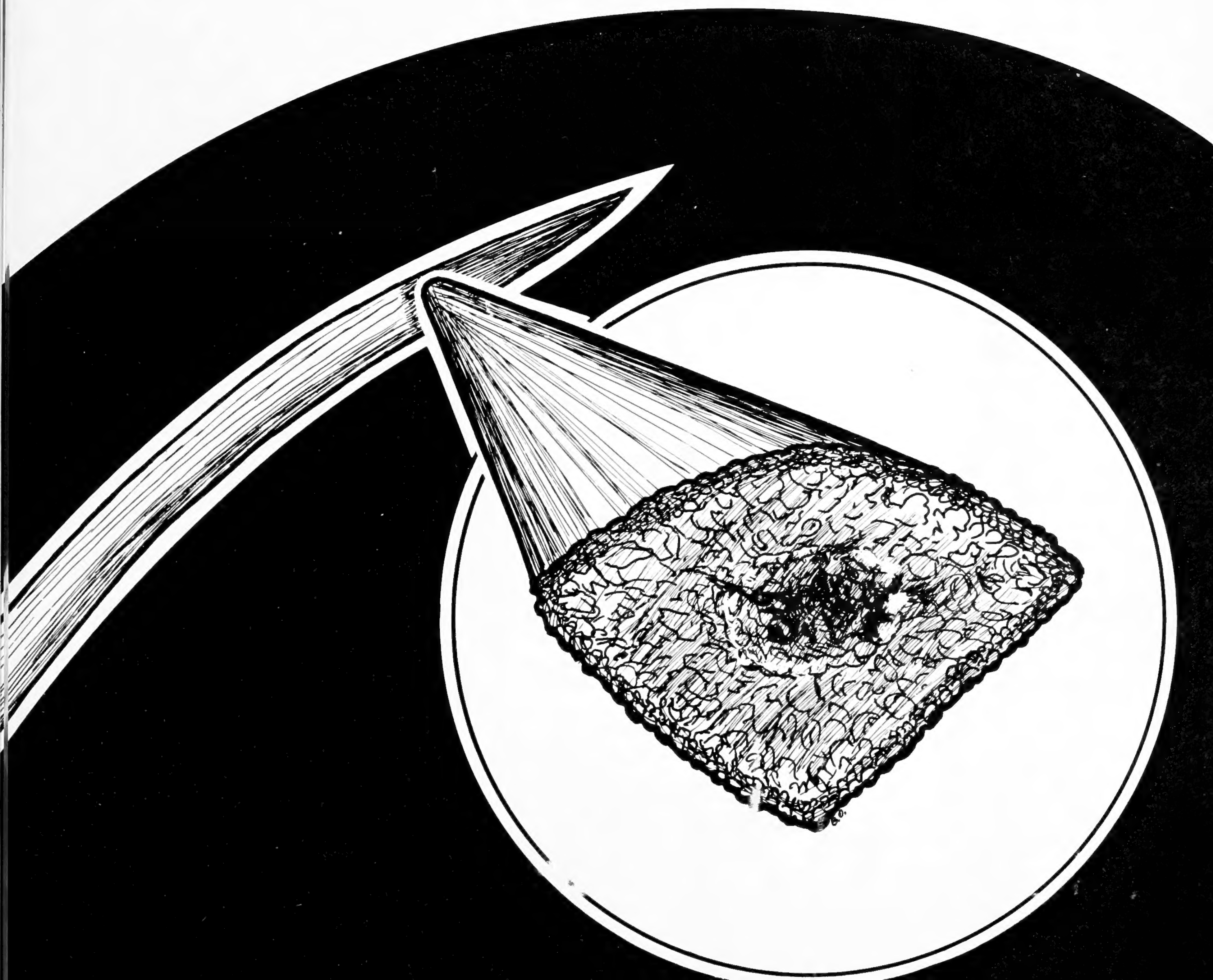
Histological Differentiation Among Abiotic Causes of Conifer Needle Necrosis

Clinton E. Carlson
Carma J. Gilligan



THE AUTHORS

CLINTON E. CARLSON is research forester with the Forestry Sciences Laboratory in Missoula, Mont. He earned his B.S. in forest science in 1964, M.S. in forestry in 1966, and Ph.D. in botany in 1978 from the University of Montana. From 1966 until 1979 he was plant pathologist with the Northern Region, USDA Forest Service, where he conducted field and laboratory studies on the effects of air pollutants on forest ecosystems. Since 1979 he has conducted silvicultural research at the Intermountain Forest and Range Experiment Station in Missoula.

CARMA J. GILLIGAN is biological technician with the Forest Pest Management Group in the Northern Region, USDA Forest Service. She has done technical field and laboratory work with effects of air pollution, insects, and biotic disease on forest ecosystems since 1970. Her work often is the basis for reports and publications concerning interrelationships between insects, diseases, and forest ecosystems.

ACKNOWLEDGMENTS

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RESEARCH SUMMARY

Histological analyses of pollutant-caused necrosis of *Pinus ponderosa* and *Pseudotsuga menziesii* current-year foliage laboratory-fumigated with hydrogen fluoride, sulfur dioxide, and ethyl mercaptan, showed that necrosis caused by phytotoxic gases can be differentiated from that induced by winter drying, drought, and salt. Hypertrophy and hyperplasia of vascular parenchyma, endodermis collapse, and intense vascular staining were characteristic of injury caused by the pollutants in both species, but were not found in needles injured by the other causes.

Similar analyses were done on necrotic 2- to 3-year-old conifer foliage collected in the field near industrial sources of fluoride, sulfur dioxide, mercaptans, and hydrogen sulfide and from specimens collected from sites known to have been injured by winter drying. Species included *Pinus contorta*, *Pinus ponderosa*, *Pseudotsuga menziesii*, *Pinus sylvestris*, and *Pinus flexilis*. As in the laboratory fumigations, winter drying injury was readily distinguished from that caused by phytotoxic gases. The internal symptoms caused by industrial fumigations were similar to those induced by gases under controlled conditions and symptoms of winter drying from field-collected specimens were similar to those simulated in the laboratory. These results differ substantially from conclusions reached in a similar study in 1973. The present study shows that histological analysis should be useful in diagnosing air pollution-induced injury and damage in coniferous forests.

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INTRODUCTION

Conifer needle necrosis and chlorosis can be induced by several agents including salt (Spotts and others 1972); winter drought (Carlson and Meyer 1973); air pollutants including fluoride, sulfur oxides, reduced sulfurs (Treshow and Pack 1970; Carlson and others 1974; Weinstein 1977); and presumably other causes. Field studies of air pollution-related injury and damage to conifers near emitting sources involve a great deal of subjectivity in the identification of cause. Even though foliar chemical analyses may indicate accumulation of a pollutant in a forest, relationships of needle abnormalities to the contaminant may remain obscure (Treshow 1969). Solberg and Adams (1956), Carlson and Dewey (1971), and Gordon (1972) suggested that light microscopy of sections of affected needles may be diagnostic. Sections were made in a transition zone defined as a ca. 0.08 in (2 mm) needle segment that includes healthy, chlorotic, and necrotic tissue. However, Stewart and others (1973) claimed that fluoride, salt, and winter damage caused virtually identical histological changes within symptomatic needles.

The purpose of this research was to determine whether abiotic causes of necrosis induce differential symptomatology in conifer needle tissue; the work involved laboratory and field studies.

METHODS

Laboratory Study

Two-year-old *Pseudotsuga menziesii* and *Pinus ponderosa* seedlings were obtained in late winter from the Forest Service nursery at Coeur d'Alene, Idaho, transplanted while dormant to 8-inch clay pots, and placed in a greenhouse. The potting medium was 70 percent nursery soil and 30 percent composted sawdust. No fertilizer was added, and pots were watered evenly every 3 or 4 days. Greenhouse temperature and humidity were recorded on a hygrothermograph throughout the experiment. The first 2 weeks following transplanting, greenhouse temperature was kept at ca. 50° F (10° C) and photoperiod at 16 hours with 550 fc (foot-candles) to minimize transplant shock. Temperature then was increased to 70° F (21° C) during the day and was allowed to drop to 50° F (10° C) at night. Bud break occurred ca. 3 weeks after transplanting. Needle development appeared normal and there was no visible evidence of transplant shock. Four months after transplanting, new foliage was fully elongated and the seedlings were physiologically active. By this time, temperature fluctuated diurnally between 50° to 91° F (10° to 33° C) and light intensity varied between 500 and 6,000 fc, depending on cloud cover. Relative humidity varied from 45 to 85 percent, averaging ca. 65 percent. Seedlings did not exhibit adverse effects due to greenhouse environmental conditions.

Several phytotoxic gases commonly emitted from industrial sources located near coniferous forests and other abiotic stresses often suggested as alternative causes of foliar injury were selected as treatments. Seedlings of each species were randomly segregated into eight groups of ten each. Each group of ten seedlings was subjected to one of the following randomly assigned treatments: control, excessive salt, drought, simulated winter drying, sulfur dioxide (SO_2), hydrogen sulfide (H_2S), ethyl mercaptan (C_2H_6S), and hydrogen fluoride (HF). All treatments except controls were administered specifically to develop foliar injury; when injury was noted seedlings were removed to the normal greenhouse environment. Treatments are detailed below.

CONTROL

Seedlings were placed in a stainless steel chamber of 18 ft^3 (.51 m^3) internal volume with plexiglass roof and windows. The chamber was refrigerated to maintain relatively constant temperatures and light was provided with a bank of fluorescent and incandescent lights emitting about 1,500 fc at tree level. Temperature was maintained at 68° to 77° F (20° to 25° C) and relative humidity at 50 to 60 percent.

Charcoal-filtered air was supplied to the chamber through a Worthington air compressor at ca. 1.77 ft^3/min (50 l/min) for 72 hours. Seedlings were then removed to the normal greenhouse environment.

SALT

Seedlings were placed in a separate watering tray and watered every 2 to 3 days for 7 weeks with a 1.2 percent (12,000 ppm) solution of NaCl according to Spotts and others (1972). Salt-free water then was applied for the duration of the experiment.

DROUGHT

Water was withheld from the seedlings for 22 days, at which time needles showed obvious visible symptoms of moisture stress. Normal watering then was resumed.

SIMULATED WINTER DRYING

A sheet of 1/2-inch (1.26-cm) thick plywood was fitted to the top of a chest-type freezer. Holes were cut large enough in the wood to allow insertion of the lower portion of the pots; the pot lip prevented the container from falling through the hole. The freezer lid was left open, the board was placed over the opening, and the pots with seedlings were inserted (fig. 1). Freezer temperature was maintained at 0° F (-18° C), effectively freezing the soil and seedling roots while the tops remained at greenhouse temperature. Soil and freezer temperatures were monitored daily. After 3 days of freezing, a small oscillating fan was placed about 8.2 ft (2.5 m) from the freezer; the fan cast a light breeze over the seedlings. Except for the relatively high greenhouse temperatures, this treatment simulated winter drought conditions. Foliar chlorosis appeared 2 days later and the treatment was discontinued. The pots then were placed in the normal greenhouse environment.

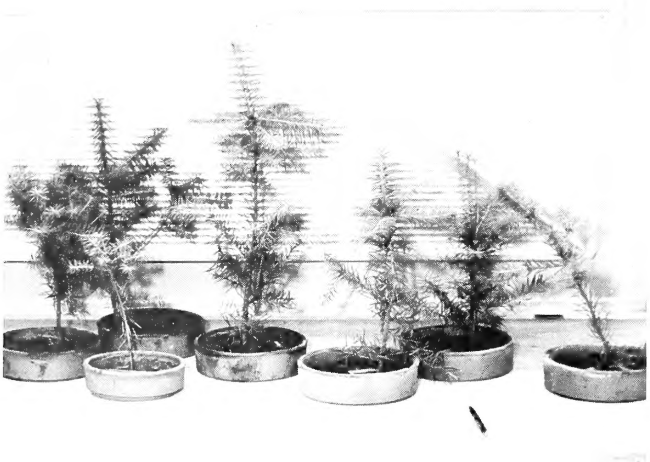


Figure 1.—Apparatus used to induce winter drying. The pot bases were subjected to freezing temperatures, freezing the soil and root systems, while the stems and needles were maintained at greenhouse temperature. A light breeze generated by a small fan directed over the seedlings completed the winter drying simulation.

SULFUR DIOXIDE

Seedlings were placed in the chamber described for the control treatment. Sulfur dioxide was obtained from Matheson Gas Co. in a pressurized tank at 1 percent SO_2 in air. Tank SO_2 was diluted with charcoal-filtered air through a mass flowmeter to achieve 5 ppm V/v in the airstream to the chamber. Air was supplied through a Worthington air compressor; flow was measured with a Matheson #605 Flowmeter. Chamber concentrations were not directly measured for sulfur dioxide nor for any of the other gas treatments. Corrections for barometric pressure and air temperatures were made as needed. Flow rate was maintained at 1.77 ft^3/min (50 l/min) until symptoms appeared on the needles, about 6 hours later. Seedlings were then removed to the normal greenhouse environment.

HYDROGEN SULFIDE

Hydrogen sulfide was obtained from Matheson in a pressurized tank at 1.20 percent in helium. The gas was delivered to the seedlings at 50 ppm for 8 hours as described for SO_2.

ETHYL MERCAPTAN

Ethyl mercaptan was obtained from Matheson at 1.28 percent in pure nitrogen and administered to the plants for 10 hours at 50 ppm as described for SO_2.

HYDROGEN FLUORIDE

Hydrogen fluoride at 113 ppm in air was obtained from Matheson and delivered to the plants at 5 ppb as described for SO_2. Injury appeared within 3 hours and the seedlings were removed from the chamber to the normal greenhouse environment.

Symptomatic current-year needles from each of the treatments were collected within 2 weeks of injury. Specimens were killed and fixed in formalin-acetic-alcohol (FAA), dehydrated through tertiary butyl alcohol, and embedded in paraffin (Johansen 1940). Serial longitudinal and transverse sections of the entire transition zone were cut to 4.72×10^{-4} inch (12 microns) thickness on a rotary microtome, stained with a Feulgen's and fast-green schedule, and observed and photographed through a phase-contrast microscope. The transition zone is the more or less gradual boundary between and including green and necrotic needle tissue. Thus, the serial sections included necrotic, chlorotic, and green tissue. This is the region in which one would expect to observe developmental symptoms of internal injury to needle tissues and to have the greatest probability of noting differences between injuries caused by different agents.

Field Study

To determine whether conifer foliage in field situations developed symptoms similar to those observed in the laboratory study, representative necrotic needles of various ages were collected from trees near seven industries known to emit phytotoxic gases and from trees in two areas damaged by winter drying. Sampling locations, major abiotic agents, sample size, and species are shown in table 1.

Necrotic needles in fluoride-polluted ecosystems were collected near an aluminum plant at Columbia Falls, Mont.; near two aluminum plants in the Rhone Valley of Switzerland; and near a phosphorus plant at Ramsay, Mont. Conifer needle samples within sulfur dioxide-polluted areas were collected near a lead smelter at Helena, Mont., and a copper smelter at Anaconda, Mont. Needles presumably injured by hydrogen sulfide were collected near a geothermal complex in California. Conifer foliage injured by a complex of sulfur dioxide, hydrogen

Table 1.—Sampling locations, major abiotic agent, species, number of trees sampled, and age of sectioned needles of field-collected specimens

Major abiotic agents	Collection location	Species	Number of trees sampled	Age of sectioned needles
				Years
Hydrogen fluoride	Anaconda Aluminum Co., Columbia Falls, Mont.	*Pinus contorta*	20	2
		Pinus ponderosa	15	2
		Pseudotsuga menziesii	10	3
	Rhone Valley, Switzerland (aluminum production)	*Pinus sylvestris*	10	2
	Stauffer Chemical Co., Ramsay, Mont. (phosphorus production)	*Pinus contorta*	2	2
		Pinus ponderosa	2	2
Sulfur dioxide	ASARCO Lead Smelter, Helena, Mont.	*Pinus ponderosa*	6	2
	Anaconda Copper Smelter, Anaconda, Mont.	*Pseudotsuga menziesii*	1	3
		Pinus contorta	5	2
		Pinus flexilis	5	2
Hydrogen sulfide	Geothermal Complex, Geyserville, Calif.	*Pinus ponderosa*	4	2,3
Sulfur dioxide, hydrogen sulfide, methyl mercaptan complex	Hoerner-Waldorf Pulp and Paper Mill, Missoula, Mont.	*Pseudotsuga menziesii*	25	2,3
		Pinus ponderosa	5	2
Winter drying	Blackfoot Valley, NE of Missoula, Mont.	*Pinus ponderosa*	2	2
	East Glacier, Mont.	*Pinus contorta*	2	2

sulfide, and methyl mercaptan was collected near a pulp and papermill at Missoula, Mont. Specimens representing winter injury were collected in the Blackfoot Valley ca. 20 miles east of Missoula, Mont., and near East Glacier, Mont.

Presence of airborne phytotoxic gases near the industrial sources was assumed. The assumption was based on the publications cited below and on personal knowledge. Air samples were not taken for pollutant analysis at the time foliage samples were collected. Fluoride emissions from an aluminum plant in northwestern Montana were described by the State of Montana (1974). Wood (1968) discussed fluorides emitted by phosphorus manufacturing facilities and the release of sulfur dioxide from copper and lead smelting operations. Hydrogen sulfide released from geothermal energy production in California was described by Miller,[1] whereas reduced sulfurs from a pulp and paper mill in western Montana were documented by Berg and others (1973). Carlson and Meyer (1973) documented the occurrence of the winter injury episode in the Blackfoot Valley in western Montana.

Field-collected specimens were 2 to 3 years old because little necrosis was found on first-year needles, whereas current-year foliage was sampled in the laboratory study. The numbers of field-collected specimens sectioned and observed by causal agent were: fluoride, 64; sulfur dioxide, 16; reduced sulfurs, 30; winter damage, 4; and control, 20. All collected specimens were embedded, sectioned, and stained as described for the laboratory study.

[1]Personal communication with Dr. Paul Miller, USDA Forest Service, Riverside, Calif.

RESULTS AND DISCUSSION

Laboratory Study

Pinus ponderosa and *Pseudotsuga menziesii* responded nearly identically within treatments and the symptoms (responses to treatment) described below apply to both species. Unless otherwise stated, all observations in the laboratory study refer to the chlorotic region within the transition zone on current-year foliage. Figures 2 through 5 show macroscopic responses of both species to control and sulfur dioxide fumigations.

CONTROL

Needles remained green and healthy throughout the experiment (figs. 2, 4). Cells of all internal tissues appeared turgid and normal, with little or no plasmolysis caused by the histological procedures (fig. 6). Plastids were dispersed within the chlorenchyma and were not granulated (table 2 and fig. 7). Xylem cells were stained light pink and living cells light greenish-blue. Staining was normally differentiated with no obvious accumulation or intensification within the vascular tissues (fig. 8).

SALT

Needles showed symptoms of salt toxicity within 15 to 20 days following the initial treatment. Chlorosis appeared at the tip, middle, or base and was independent of species or individual. Usually the transition zone was diffuse, not distinct. In all cases, symptoms intensified until the trees were dead, even though fresh water replaced the salt treatments soon after the onset of symptoms. Mesophyll chlorenchyma plasmolyzed extensively and most plastids were destroyed (figs. 9, 10). Endodermal cells in the region of plasmolyzed mesophyll remained turgid and intact but xylem, phloem, and transfusion parenchyma collapsed (table 2). Phloem elements virtually disintegrated, and there was no obvious accumulation of stain within the transition zone (fig. 10).

DROUGHT

Chlorosis most often appeared first at the needle base and progressed acropetally. No distinct transition zone was formed. Mesophyll cells collapsed but did not plasmolyze as in the salt treatment (table 2). All other living cell types collapsed except the endodermis (fig. 11). No obvious accumulation of stain occurred within the transition zone.

WINTER DRYING

The syndrome for winter drying (table 2) was virtually the same as for drought except that injury appeared much sooner, within 3 to 5 days (figs. 12, 13).

SULFUR DIOXIDE

Chlorosis appeared near or at the tips within 3 to 8 hours following fumigation and an abrupt, distinct transition zone developed (figs. 3, 5). Sporadic collapse of the mesophyll occurred, and plastids clumped near the plasmalemma. Endodermis cells in contact with a necrotic mesophyll chlorenchyma collapsed (figs. 14, 15). Xylem, phloem, and transfusion parenchyma and phloem elements showed extensive hypertrophy and hyperplasia. Epithelial cells hypertrophied, occluding the resin canals (fig. 16). An intense, purple-red stain developed in the vascular tissues and extended basipetally into the region of nondamaged mesophyll (table 2).

HYDROGEN FLUORIDE, HYDROGEN SULFIDE, AND ETHYL MERCAPTAN

The internal syndrome induced by these pollutants was indistinguishable from that caused by SO_2 (table 2 and figs. 17–19). However, H_2S caused necrosis to only the needle tips, whereas F^- and C_2H_6S injury initially appeared slightly below the tips and progressed acropetally and basipetally. Also, tracheid cell walls were stained yellowish in needles injured by H_2S, unlike the light red in needles injured by the other gases.

Field Study

Control specimens were histologically similar to those of the laboratory study (fig. 20). Similarly, symptoms in the transition zones of field-collected needles within polluted areas were identical with symptoms induced by gaseous pollutants in the greenhouse study (figs. 23–28). Plastids clumped in the mesophyll parenchyma; endodermis collapsed when in contact with damaged mesophyll; and phloem, xylem, and transfusion parenchyma and epithelial cells divided excessively and became abnormally large. Resin ducts were occluded by the hypertrophy of epithelial cells. An intense purple-red stain developed in the vascular tissue extending well into the area of nondamaged mesophyll. The total syndrome was similar regardless of pollutant or species; for example, it was not possible to differentiate fluoride injury from SO_2, and all species and needle ages responded similarly.

Winter drying, however, was dissimilar to pollutant injury; this difference also was observed in the laboratory study. Mesophyll collapsed, endodermis remained turgid even when in contact with collapsed, necrotic mesophyll, and no hypertrophy or hyperplasia occurred in the parenchyma (figs.21, 22). Epithelial cells in winter-dried specimens were hypertrophied, often occluding the resin canals. No intense stain developed in the vascular cylinder such as occurred with gas-injured specimens; the symptoms were similar among the different species and needles of various ages.

Histological differentiation of needle necrosis has not been clearly defined. Solberg and others (1955) and Solberg and Adams (1956) showed that HF and SO_2 disrupted vascular tissues and caused hypertrophy and hyperplasia of vascular parenchyma. They indicated that HF injury could be distinguished from SO_2. Evans and Miller (1972, 1975) stated that SO_2, suspected winter injury, and ozone injury could be distinguished histologically in that SO_2 disrupted and dissolved cytoplasmic constituents of all needle tissues, whereas ozone injured only the plicate parenchyma and winter fleck caused abnormalities within the phloem and transfusion cells. Their sections were taken adjacent to necrotic lesions and not within a transition zone as described in this paper. Also, their winter injury was suspected to be direct cold injury and not of the drying type. Stewart and others (1973) were not able to distinguish between symptoms of SO_2, HF, and winter injury. They observed hypertrophy of phloem cells and mesophyll parenchyma cells when natural senescence, drought, and fluoride were causes of necrosis. Consequently, Stewart and others (1973) saw little value in the use of histological interpretations to differentiate among various environmental stresses. Conversely, biotic causes usually are easily diagnosed histologically by signs of the causal organism.

Table 2.—Qualitative comparison of experimentally induced abiotic stresses on various *Pseudotsuga menziesii* and *Pinus ponderosa* needle tissues[1]

Treatment	External symptomatology	Mesophyll	Endodermis	Transfusion parenchyma	Xylem parenchyma	Phloem	Phloem parenchyma	Epithelial cells	Staining patterns[2] in vascular tissue
Control	Needles remained deep green	Fully turgid, plastids not clumped, dispersed	Intact, turgid	Turgid	Turgid	Turgid	Turgid	Turgid	No deep staining, xylem light pink, phloem light green, stain homogeneous throughout
Salt, 12,000 ppm in tap-water	Chlorosis of base, middle, or tip within 15–20 days, zone between necrotic and green tissue not distinct	Extreme plasmolysis, chloroplasts destroyed	Inact, turgid	Collapsed	Collapsed	Disintegrated	Collapsed	Collapsed	Normal, no particular concentration of stain
Drought, water withheld	Chlorisis and necrosis within 15–25 days, often progressing acropetally from base. Diffuse, no distinct banding. Needles twist.	Collapsed, but not plasmolyzed	Intact, turgid	Collapsed	Collapsed	Collapsed	Collapsed	Collapsed	Normal, no particular concentration of stain
Winter drying, roots frozen, 25–30°C, slight breeze	Same as drought, but injury occurs within 3–5 days	Collapse	Intact, turgid	Collapsed	Collapsed	Collapsed	Collapsed	Collapsed	Same as Salt and Drought
Sulfur dioxide, 5 ppm	Chlorosis at tips or slightly proximal to tip within 3–8 h, abrupt transition between green and chlorotic tissue	Clumping of plastids, some collapse	Collapsed where mesophyll is necrotis	Hypertrophy and hyperplasia	Hypertrophy and hyperplasia	Hypertrophy, hyperplasia, & collapse	Hypertrophy and hyperplasia	Hypertrophied, occluding resin canal	Intense purple-red and purple-blue extending basipetally into area of non-damaged mesophyll
Fluoride, 5 ppb	Chlorosis slightly below tips within 5–6 h, progressing acropetally. Abrupt transition zone	Clumping of plastids, little collapse	Collapsed where mesophyll is necrotic	Hypertrophy and hyperplasia	Hypertrophy and hyperplasia	Hypertrophy and hyperplasia	Hypertrophy and hyperplasia	Hypertrophied, resin canals occluded	Same as SO_2
H_2S, 50 ppm	Well defined tip chlorosis within 20 h, necrosis limited to tips, transition zone abrupt.	Same as SO_2, HF	Same as SO_2, HF	Same as SO_2 and HF	Same as SO_2 and HF	Same as SO_2 and HF	Same as SO_2 and HF	Same as SO_2 and HF	Yellow tones in cell walls, intense purple-red in lumens, extending basipetally into area of non-damaged mesophyll
C_2H_6S, 50 ppm	Tip chlorosis within 20–15 h, transition zone abrupt.	Same as SO_2, HF	Same as SO_2 and HF	Same as SO_2 and HF	Same as SO_2 and HF	Same as SO_2 and HF	Same as SO_2 and HF	Same as SO_2 and HF	Same as SO_2 and HF

[1]All observations were made within the transition zone, a 2 mm segment including the necrotic, chlorotic, and green portions of injured needle. Generally, the transition zone is chlorotic.
[2]Feulgen's and fast green schedule.

Figure 2.—Lab study. Control ponderosa pine seedling after treatment with clean air in fumigation chamber. Current-year foliage remained green; no necrosis or chlorosis was observed.

Figure 3.—Lab study. Ponderosa pine seedling after treatment with 5 ppm SO_2 for 6 hours. All current foliage developed necrosis.

Figure 4.—Lab study. Control Douglas-fir seedling. Similar to ponderosa pine, current-year foliage remained green and appeared healthy.

Figure 5.—Lab study. Douglas-fir foliage after fumigation with 5 ppm SO_2 for 6 hours. Note the well-defined tip necrosis. Needle segments for histological study were taken from the transition zone, which included green, chlorotic, and necrotic tissue. Serial longitudinal and transverse thin sections 4.72×10^{-4} in (12 microns) thick then were made through the entire transition zone. Histological interpretations were made from these sections.

Figure 2

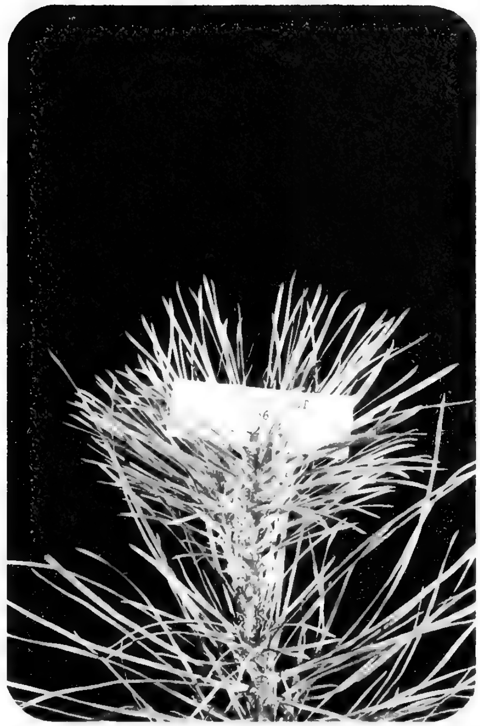


Figure 3

Figure 4

Figure 5

Figure 6.—Lab study. Control, ponderosa pine, longitudinal section, X300. Endodermis (EN), vascular parenchyma (VP), phloem (P), and xylem (X) are fully turgid. Hypertrophy and hyperplasia of parenchymatous tissue have not occurred here, but do in needles affected by phytotoxic gases. Current-year foliage.

Figure 7.—Lab study. Control, ponderosa pine, transverse section, X300. Note position of epidermis (ED), hypodermis (HY), mesophyll parenchyma (MP), and resin canal (RC). Presence of numerous dispersed plastids in turgid mesophyll cells and nonhypertrophied epithelial cells (EP) in the resin canal are indicative of a healthy needle. Current-year foliage.

Figure 8.—Lab study. Control, ponderosa pine, transverse section, X300. Note position of phloem (P), vascular parenchyma (VP), xylem (X), endodermis (EN), and mesophyll (MP). Endodermis is turgid and phloem and vascular parenchyma are not deeply stained nor hypertrophied. Mesophyll is turgid and packed with plastids. Current-year foliage.

Figure 9.—Lab study. Salt injury, ponderosa pine, longitudinal section, X300. Mesophyll parenchyma (MP) collapsed, but the endodermis (EN) remained turgid even when in contact with necrotic mesophyll cells. Note the junction between necrotic mesophyll and healthy endodermis (arrow). Current-year foliage.

Figure 10.—Lab study. Salt injury, ponderosa pine, transverse section, X300. This section is from the necrotic portion of the transition zone. Parenchymatous tissues including mesophyll (MP), vascular parenchyma (VP), and phloem (P) collapsed. Even in this condition of extreme injury, the endodermis (EN) is, for the most part, somewhat turgid. Current-year foliage.

Figure 11.—Lab study. Drought, ponderosa pine, longitudinal section, X300. Mesophyll (MP) collapsed but endodermis (EN) in contact with necrotic mesophyll remains turgid. Current-year foliage.

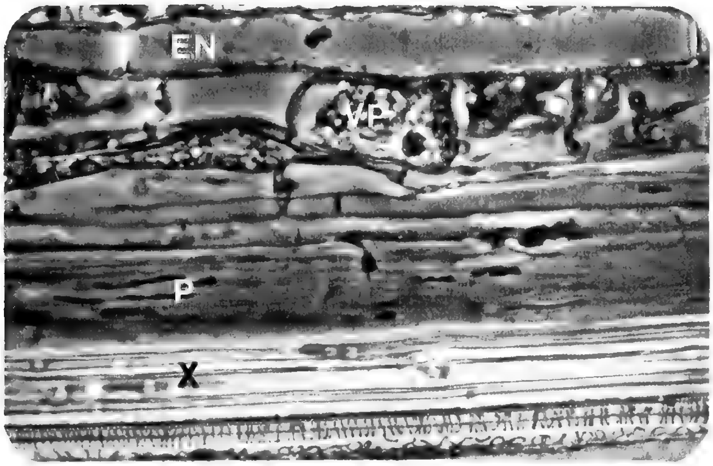
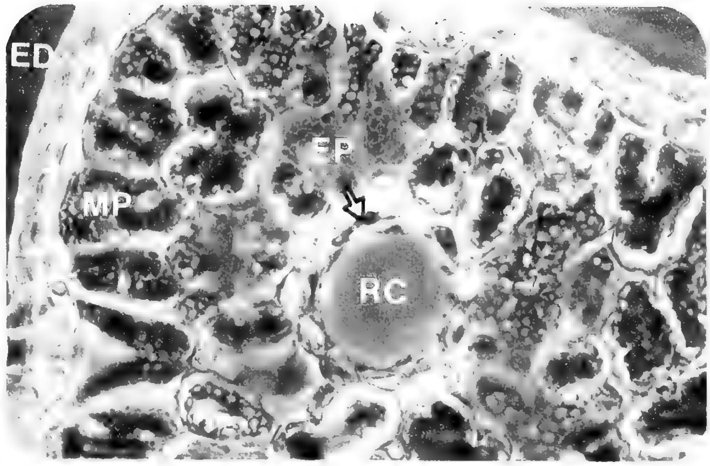


Figure 6



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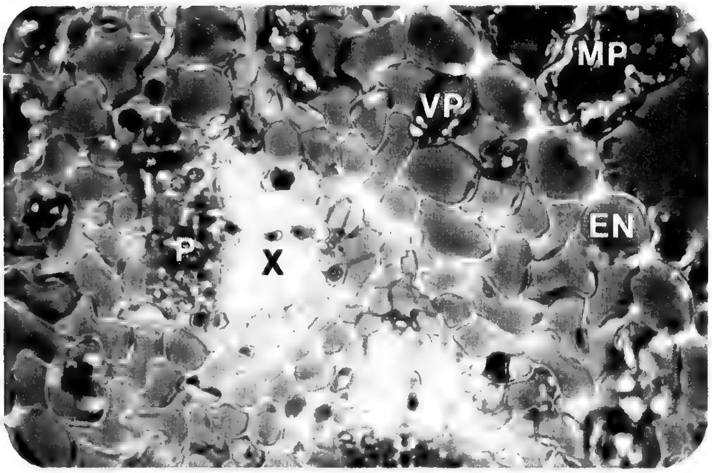


Figure 8

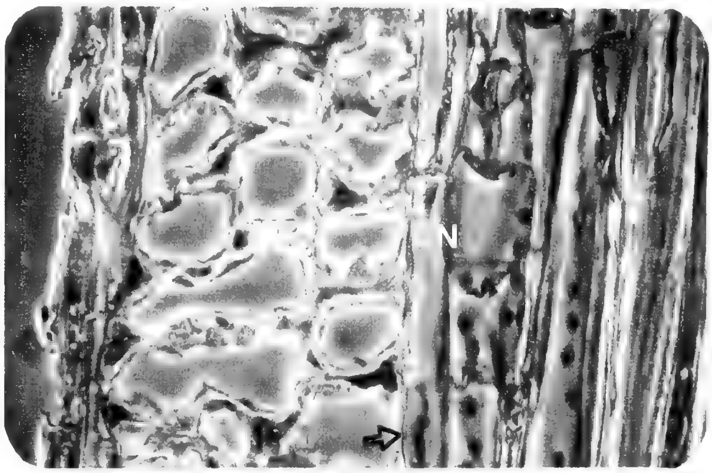


Figure 9

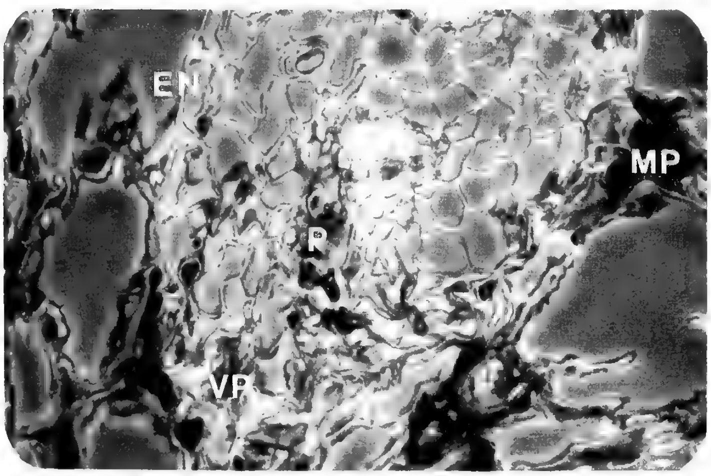


Figure 10

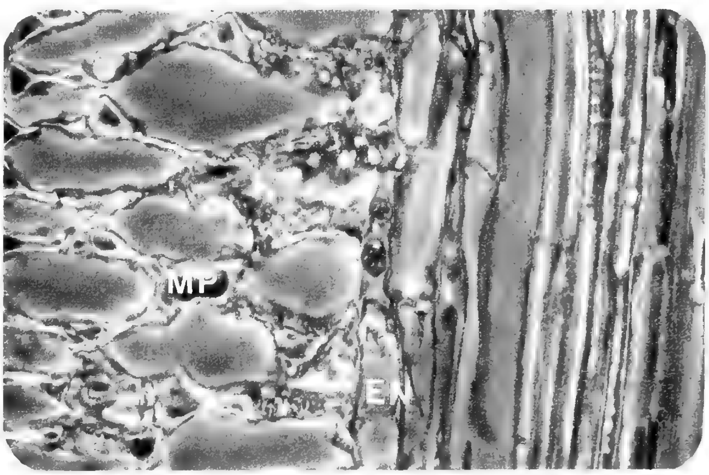


Figure 11

Figure 12.—Lab study. Winter drying, ponderosa pine, longitudinal section, X300. Mesophyll (MP) collapsed, but endodermis (EN) remained turgid. Note similarity between injury by salt, drought, and winter drying (figs. 9-12). Current-year foliage.

Figure 13.—Lab study. Winter drying, ponderosa pine, transverse section, X300. Mesophyll (MP), vascular parenchyma (VP), and phloem (P) collapsed while endodermis remains turgid. No deep staining occurred in the vascular tissues. Current-year foliage.

Figure 14.—Lab study. Sulfur dioxide, ponderosa pine, longitudinal section, X300. Mesophyll (MP) collapsed and endodermis (EN) in contact with collapsed necrotic mesophyll also collapsed. Note the intense reddish staining in the vascular bundle (within the endodermis) extending basipetally into the region of noninjured mesophyll. Note the hypertrophy (excessive cell enlargement [HT]) and hyperplasia (excessive cell division [HP]) that occurred in the vascular parenchyma, causing the vascular bundle to swell. These three symptoms, endodermis collapse, intense vascular stain, and swelling, are characteristic of injury induced by phytotoxic gas, but not by other causes. Compare with salt, drought, and winter drying (figs. 9-13). Current-year foliage.

Figure 15.—Lab study. Sulfur dioxide, ponderosa pine, transverse section, X300. This section is from the green end of the transition zone. Vascular parenchyma (VP) hypertrophied and the characteristic deep reddish stain pervaded the vascular tissues. Mesophyll (MP) has not collapsed. Phloem (P) has been destroyed. Current-year foliage.

Figure 16.—Lab study. Sulfur dioxide, ponderosa pine, transverse section, X300. Epithelial cells (EP) hypertrophied and occluded the resin canal (RC). Mesophyll (MP) is not noticeably affected. Hypertrophy of epithelial tissue commonly occurs in needles injured by phytotoxic gases, but also is found in needles injured by winter drying and drought. It is not distinctive for phytotoxic gases. Current-year foliage.

Figure 17.—Lab study. Hydrogen sulfide, ponderosa pine, longitudinal section, X300. Collapsed endodermis (EN), deep-red vascular staining, and hypertrophy of vascular parenchyma (VP) are evident. These symptoms are similar to those caused by SO_2. Current-year foliage.

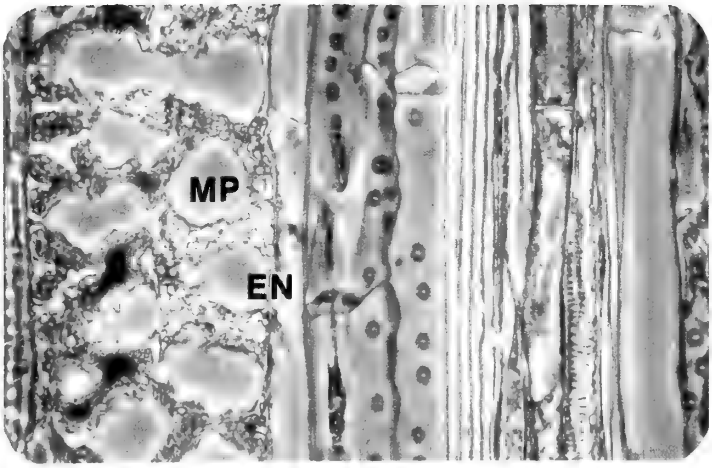


Figure 12

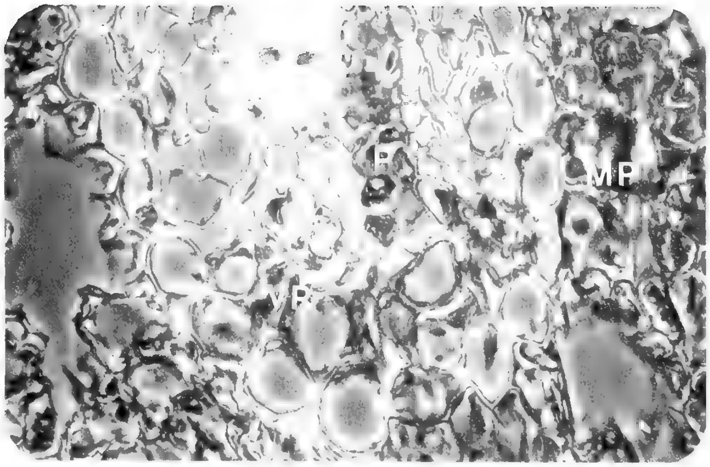


Figure 13

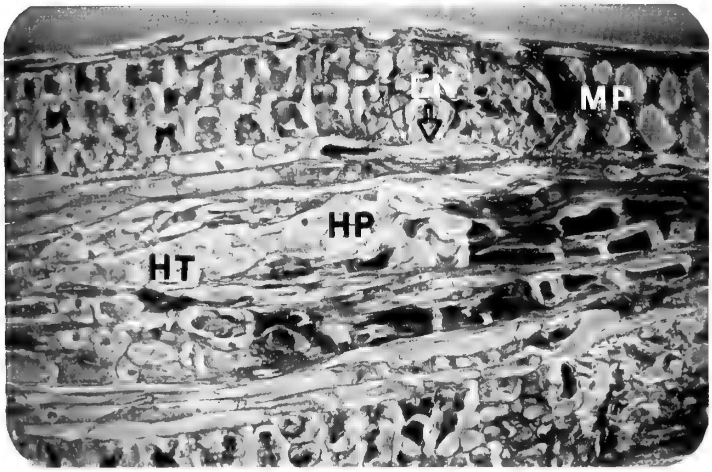
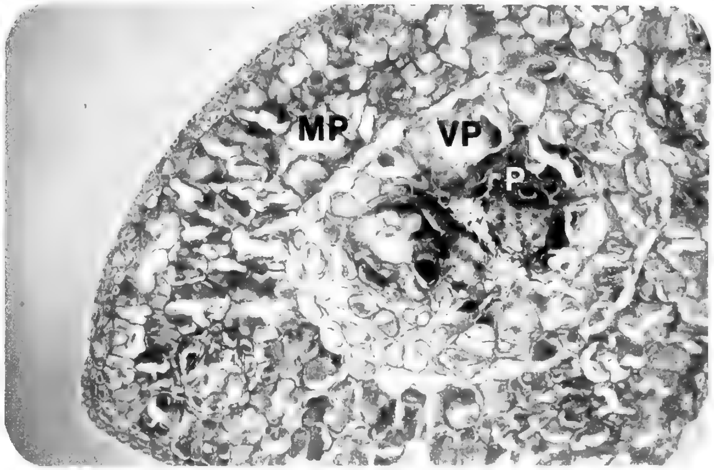


Figure 14



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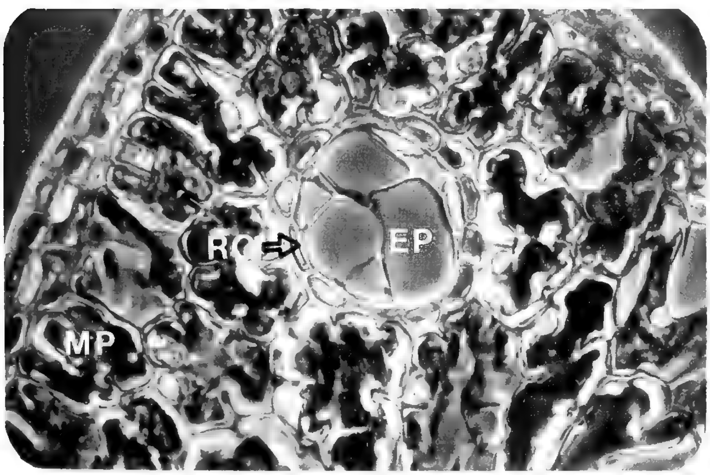

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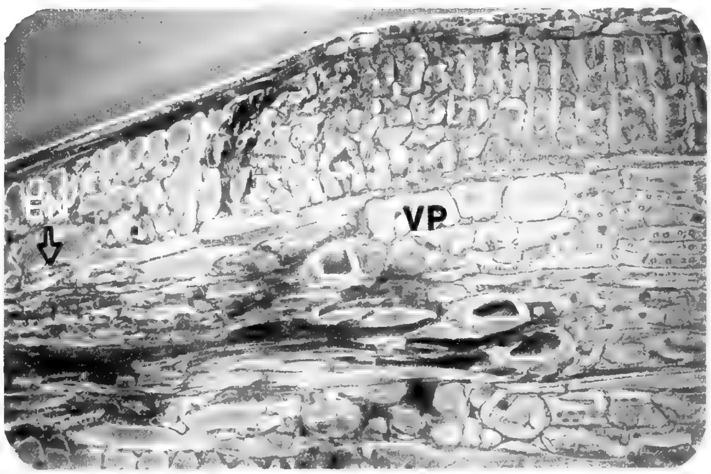


Figure 17

Figure 18.—Lab study. Ethyl mercaptan, ponderosa pine, longitudinal section, X300. Symptoms are similar to those caused by sulfur dioxide. Note hypertrophied vascular parenchyma (VP), intense reddish staining in vascular bundle, and endodermis (EN) collapse in contact with necrotic mesophyll (MP). Current-year foliage.

Figure 19.—Lab study. Hydrogen fluoride, Douglas-fir, longitudinal section, X300. Note collapse of endodermal cell (EN) in contact with necrotic mesophyll (MP). Also, the vascular bundle is deeply stained. Symptoms are similar to those caused by sulfur dioxide, hydrogen sulfide, and ethyl mercaptan in ponderosa pine needles. Compare with figures 14–18. In this experiment, the different symptoms induced by various treatments in ponderosa pine needles were identical to those induced in Douglas-fir needles. Current-year foliage.

Figure 20.—Field study. Control, ponderosa pine, transverse section, X125. Note position of mesophyll (MP), endodermis (EN), phloem (P), xylem (X), and vascular parenchyma (VP). The only difference between this needle and the control needle of the lab study (fig. 8) is that the phloem and xylem are better developed. All needles shown for field study were 2 years old.

Figure 21.—Field study. Winter drying, ponderosa pine, longitudinal section, X25. Note position of epidermis (ED), mesophyll (MP), resin canal (RC), endodermis (EN), vascular parenchyma (VP), phloem (P), and xylem (X). The collapsed mesophyll (arrow) identifies the necrotic part of the needle. No intensive, reddish staining occurred in the vascular bundle nor did transfusion parenchyma undergo hypertrophy and hyperplasia.

Figure 22.—Field study. Winter drying, ponderosa pine, longitudinal section, X125. Note collapsed mesophyll (MP). Endodermis (EN) in contact with collapsed mesophyll is turgid, healthy, and vascular parenchyma (VP) is normal. This section included some epithelial (EP) tissue that also appears normal.

Figure 23.—Field study. Sulfur dioxide, ponderosa pine, longitudinal section, X25. Deeply stained vascular tissue (VT), collapsed mesophyll (MP), and necrotic endodermal cells (EN) occurred. This is similar to specimens injured by sulfur dioxide in the lab study.

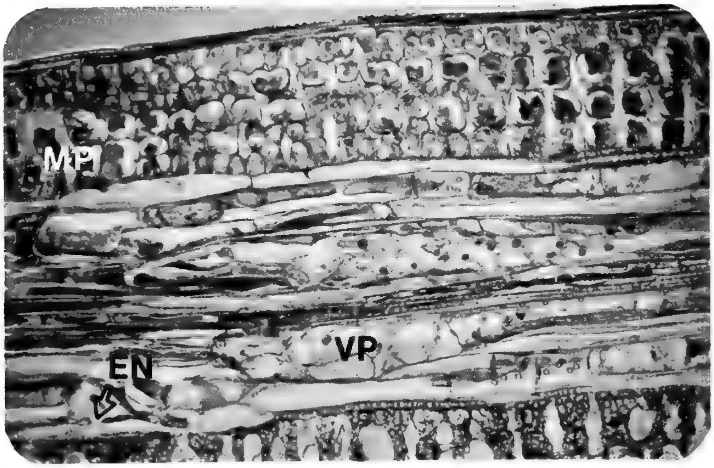
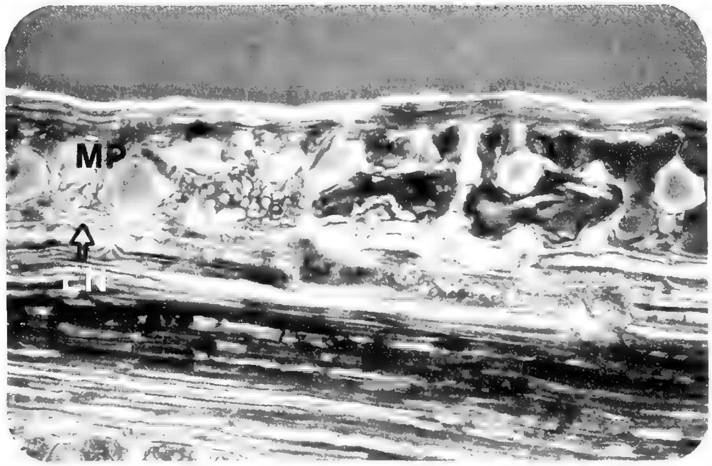


Figure 18



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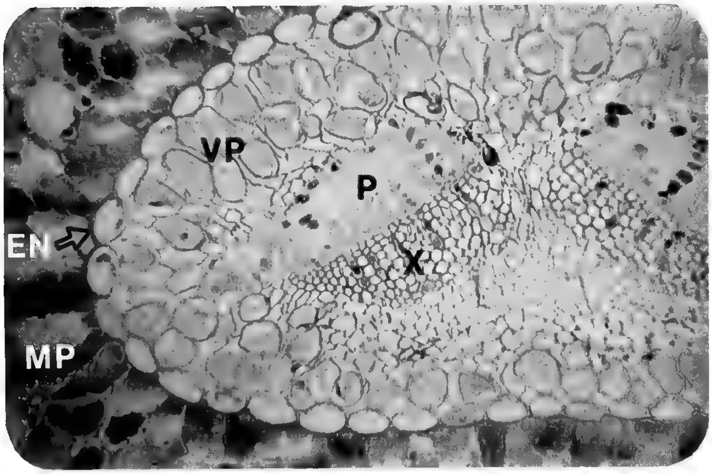
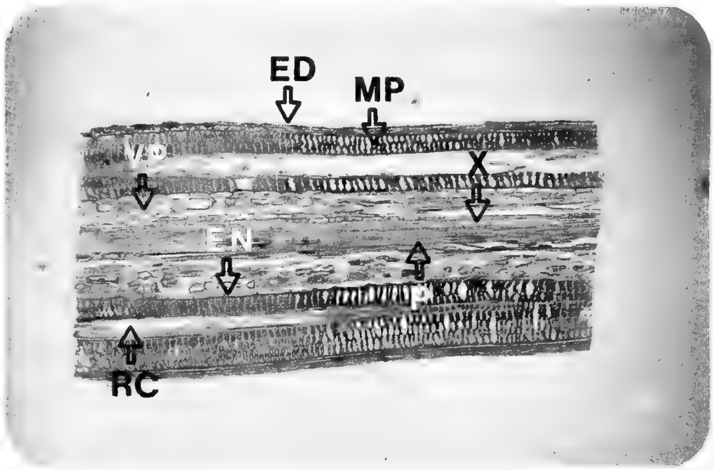


Figure 20



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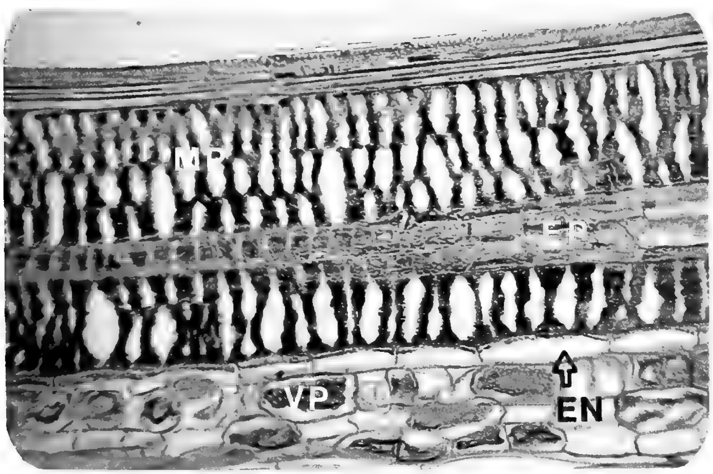

Figure 22

Figure 23

Figure 24.—Field study. Sulfur dioxide, ponderosa pine, transverse section, X125. Deep reddish stain (arrow) and necrotic endodermis (EN) in contact with necrotic mesophyll (MP) typify injury by phytotoxic gases.

Figure 25.—Field study. Hydrogen sulfide, ponderosa pine, longitudinal section, X125. Note necrotic endodermis (EN), intense stain in vascular tissue (VT), and hypertrophy of vascular parenchyma (VP).

Figure 26.—Field study. Hydrogen sulfide, ponderosa pine, transverse section, X125. This section is from the greenish portion of the transition zone. Note destruction and heavy staining in the vascular tissues (VT). Note also the hypertrophy and hyperplasia in the vascular parenchyma (VP).

Figure 27.—Field study. Hydrogen fluoride, Scotch pine, longitudinal section, X125. These specimens were obtained from the Rhone Valley in Switzerland. Characteristic of injury caused by fluoride and other phytotoxic gases, endodermal cells (EN) in contact with necrotic mesophyll (MP) are necrotic, the vascular cylinder (VC) is deeply stained, and vascular parenchyma (VP) has hypertrophied. Compare with figures 14 through 19 and 23 through 26.

Figure 28.—Field study, hydrogen fluoride, Scotch pine, transverse section, X125. This specimen also is from the Rhone Valley in Switzerland. The section was cut in the greenish portion of the transition zone. Notice the deep staining of vascular parenchyma (VP).

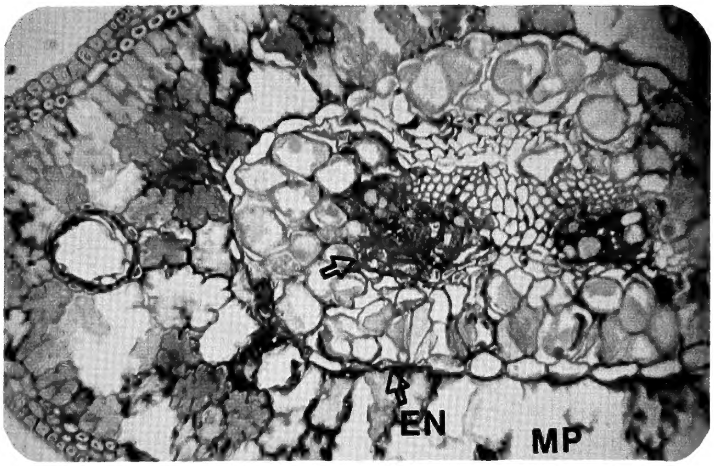

Figure 24

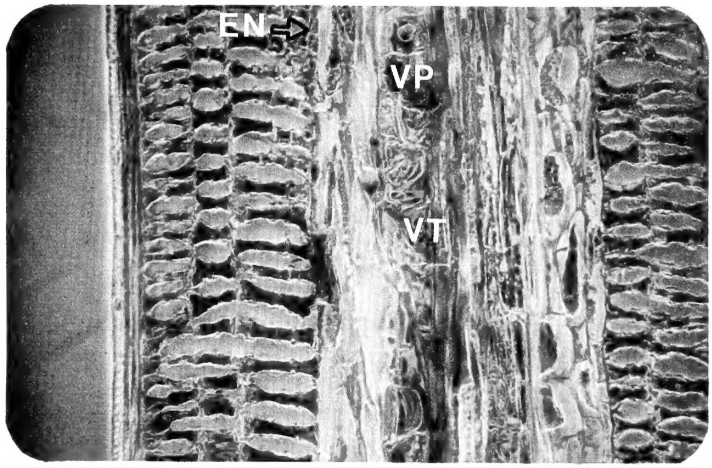

Figure 25

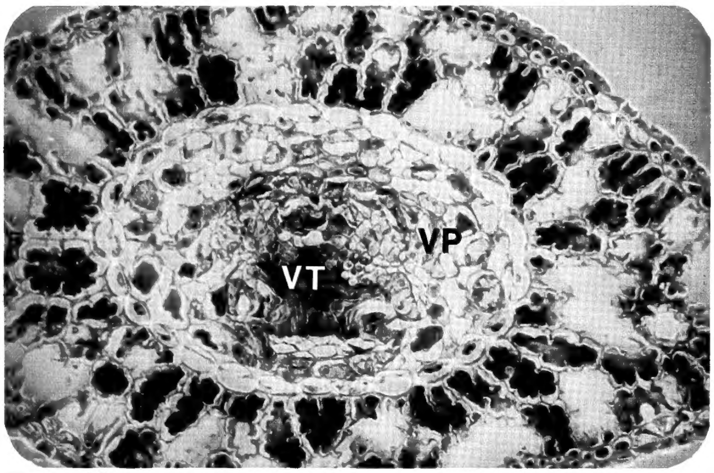

Figure 26

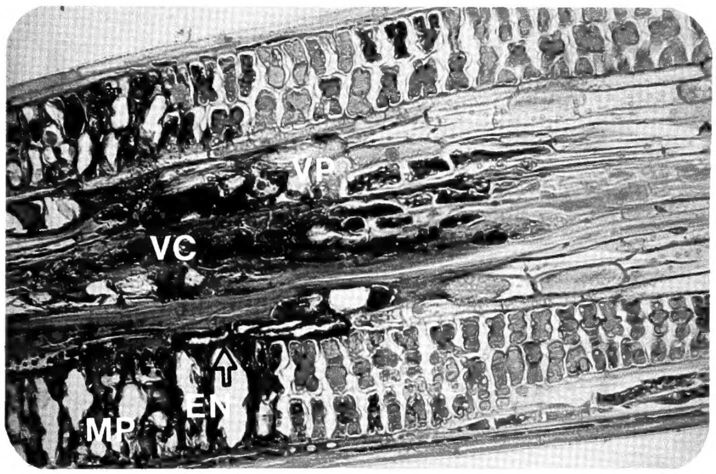

Figure 27

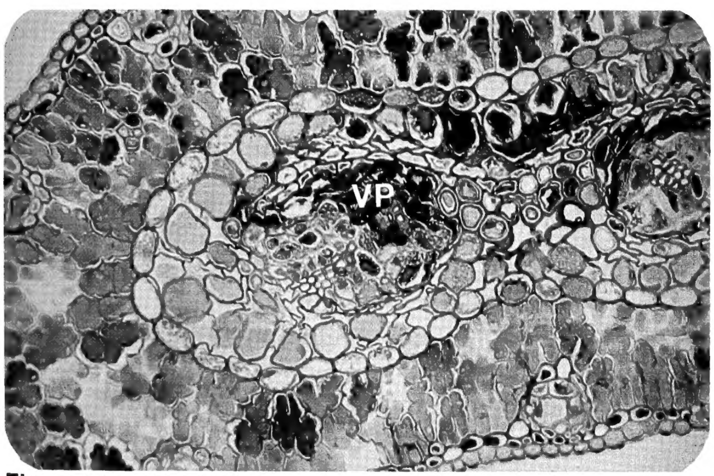

Figure 28

Our studies show that needle necrosis caused by phytotoxic gases can be differentiated microscopically from necrosis caused by other abiotic agents. The controlled greenhouse study showed that symptoms of gas-caused needle necrosis can be distinguished from symptoms of selected nongaseous causes. Phytotoxic gases caused extensive damage to parenchymatous tissues, especially within the vascular system, but it was not possible to distinguish among symptoms caused by various gases. Winter drying and drought caused mesophyll damage, but the tissues of the vascular system were not affected. Endodermal cells in contact with necrotic mesophyll became necrotic when phytotoxic gas was the cause, but not when damage was induced by winter drying or normal drought; necrotic endodermal cells could result from acid hydrolysis. The mechanisms of endodermis susceptibility to phytotoxic gas, but resistance to winter damage, salt, and drought is biologically interesting and should be investigated. The deep staining of vascular tissue in the transition zone noted in the gas fumigations could have been induced by cytoplasmic dissolution. Evans and Miller (1975) noted that sulfur dioxide decreased the intensity of stain where cytoplasmic dissolution was extensive. We observed the same in the necrotic areas. However, in the chlorotic part of the transition zone, leakage of cytoplasmic contents from lysed cells into the intercellular spaces could account for the general deep staining observed in our study.

The histological interpretations of field-collected specimens were similar to those of the laboratory investigation; specimens collected from Switzerland, Montana, and California near known sources of phytotoxic gases exhibited the pollutant syndrome, but it was not possible to distinguish between pollutants. Winter-induced needle necrosis collected from areas of known winter drying was similar histologically to the laboratory-induced winter drying. Studies by EPA (1971), Gordon (1972), Gordon and Tourangeau (1975), Carlson and Dewey (1971), Carlson and others (1974), and Gordon and others (1976) support these findings. In studies with tip-burned field-collected Austrian pine needles, Maiello and others (1972) determined that sulfur dioxide caused distortion of endodermis and transfusion tissues and that this disruption of vascular tissues extended about 0.02 in (0.5 mm) into the region of healthy mesophyll. Results of our studies agree.

Solberg and Adams (1956), Gordon (1972), and Gordon and Tourangeau (1975) believed they could distinguish between injury caused by sulfur oxides and fluoride. Vascular tissues were disrupted in the area of healthy and necrotic mesophyll when fluoride was causal, whereas vascular tissues appeared normal even in the region of necrotic mesophyll when sulfur dioxide was the agent. We did not observe this either in the controlled fumigation or in the field-collected specimens. It seems unlikely that plant tissues would have separate response syndromes to individual gases. However, it is plausible that responses would differ between such different agents as gases versus drought or salt, as suggested by our work.

The simulated winter injury treatment subjected physiologically active, succulent young needle tissue to relatively severe stress. Natural winter drying occurs to older foliage (8 to 10 months) that likely is in a subdued physiological state. Thus, the simulated treatment can be interpreted as severe. However, notwithstanding these treatment differences, the histological effects of field- and laboratory-induced stress were similar, indicating similarity between physiological and morphological responses.

The gases were administered at high concentrations, presumably much higher than one would expect to find in field situations. Also, the gas concentrations were not monitored in the exposure chamber, but were inferred from flow input. We were not interested in testing effects of differential gas concentrations; rather, we wished to assure the development of needle necrosis and to compare between treatments. Histological reactions to our dosages may represent responses only to short-term acute fumigations and may not be representative of actual field situations. However, histological symptoms in field-collected specimens were similar within treatment to those in needles with experimentally induced necrosis. It is likely, but not known, that necrosis on needles from field locations was caused by longer-term, lower concentration of pollutant. If so, the continuity between symptoms observed in field and laboratory specimens suggests that the histological reactions represent a biochemical-morphological reaction not entirely dependent on gas concentration.

Our field study did not include either drought- or salt-caused needle necrosis. However, there is little reason to believe that field-induced symptoms would differ significantly from our greenhouse work.

Finally, the concept that needle necrosis caused by phytotoxic gases can be separated from other selected abiotic agents is well supported in that differences observed in the laboratory experiment also were observed in corresponding field collections.

SUMMARY AND CONCLUSIONS

Phytotoxic gases cause histological symptoms in Douglas-fir and ponderosa pine needles distinct from those induced by winter damage, drought, or salt. The symptoms caused by gaseous pollutants under controlled conditions are:

1. Collapse of endodermis in contact with collapsed mesophyll.
2. Hypertrophy and hyperplasia of vascular parenchyma.
3. Deep staining of vascular tissue, extending basipetally into the region of healthy mesophyll.

Winter damage, drought, and salt induce the following symptoms:

1. Mesophyll cells collapse, but endodermis is not affected, even when in contact with necrotic mesophyll.
2. Vascular parenchyma collapse; hypertrophy and hyperplasia are not evident.
3. Deep staining of vascular tissues does not occur.

Symptoms in field-collected specimens representative of a variety of conifers were similar for similar causal agents.

Identification of the cause of conifer injury and related forest damage near sources of phytotoxic air emissions may be confounded by insects, disease, weather, or other abiotic agents. Affected trees usually exhibit foliar chlorosis and necrosis in response to causal factors. This study demonstrates that through histological procedures these causal factors may be distinguished. When used in conjunction with ambient air quality data, emission data, foliar chemical analyses, and observations of the status of biotic plant pathogens and foliage-feeding insects, histological observations should strengthen the diagnosis of cause in damage surveys done near polluting industries.

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Symptoms induced by phytotoxic gases within conifer needles can be differentiated histologically from those caused by other abiotic agents including winter drying, drought, and salt. However, it is not possible to differentiate among symptoms caused by hydrogen fluoride, sulfur dioxide, ethyl mercaptan, and hydrogen sulfide. Phytotoxic gases cause hypertrophy and hyperplasia of vascular parenchyma, endodermis collapse, and intense vascular staining. The other abiotic agents induce mesophyll collapse with little or no observable effects on vascular tissues. Histological analyses should be useful in diagnosis of air pollution-induced injury and damage in coniferous forests.

KEYWORDS: phytotoxic gases, winter injury, conifer needles, symptoms, histology, air pollution, diagnosis, needle necrosis

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